Postprint: Uptake and Distribution of Different Nitrogen Forms in Two Legume Species and Their Organs

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Abstract

Legume plants occur extensively in nitrogen-deficient desert ecosystems, serving as the central source of available nitrogen and as important pioneer species in these regions. However, studies on nitrogen absorption and utilization by legume plants in desert ecosystems have been rarely reported to date. This study selected Astragalus flexus and Astragalus arpilobus, which are widely distributed in the Gurbantunggut Desert, as research subjects. Three different nitrogen forms (15N-NH4 +, 15N-NO3 -, 15N-Glycine) were added to soil layers at 0-5 cm and 5-15 cm to investigate the absorption and allocation strategies of the two plants and their organs for different nitrogen forms. The results showed that: (1) In different soil layers, both plants preferentially absorbed nitrate nitrogen, with the highest uptake rates of nitrate nitrogen by Astragalus flexus and Astragalus arpilobus being 3.26 and 2.59 g ·g–1 ·h–1, respectively. (2) In different soil layers, there were significant differences in the absorption and allocation of different nitrogen sources among plant organs ($P < 0.05$). The 15N uptake by roots of Astragalus flexus was greater than that by roots of Astragalus arpilobus, and the three different nitrogen forms were mainly allocated to leaves. (3) In different soil layers, the contribution rates of different nitrogen sources to the two plants followed the order: 15N-NO3 - > 15N-Glycine > 15N-NH4 +, with the contribution rate of nitrate nitrogen to nitrogen absorption by Astragalus flexus ranging between 37% ~ 41%, while that for Astragalus arpilobus reaching up to 45%. (4) There were significant differences in the recovery rates of different nitrogen forms among plant organs ($P < 0.05$). In the 0-5 cm soil layer, the recovery rates of nitrate nitrogen by plant organs followed the order: leaf > stem > root, while in the 5-15 cm soil layer, Astragalus flexus showed the pattern: leaf > root > stem. Overall, in the Gurbantunggut Desert ecosystem, different life-form legume plants exhibit both consistency and differences in nitrogen absorption and allocation capacities, which are influenced by different soil depths and nitrogen forms. These results provide a theoretical

basis for nitrogen absorption, utilization, and allocation of legume plants in arid and semi-arid regions of Xinjiang.

Full Text

Preamble

Studies on the Absorption and Distribution of Different Nitrogen Forms in Two Legume Species and Their Organs

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Abstract: Legumes are abundant in nitrogen-deficient desert ecosystems, where they serve as central providers of available nitrogen and represent important pioneer species. However, studies on nitrogen uptake and utilization by leguminous plants in desert ecosystems remain scarce. This study selected *Astragalus flexus* and *Astragalus arpilobus*, two widely distributed species in the Gurbantunggut Desert, and added three different nitrogen forms (^{15}N-NH$_4{}^+$, ^{15}N-NO$_3{}^-$, ^{15}N-Glycine) to soil layers at 0–5 cm and 5–15 cm depths to investigate the absorption and allocation strategies of different nitrogen forms in both species and their organs. The results showed that: (1) In both soil layers, both plant species preferred nitrate nitrogen, with maximum absorption rates of 3.26 and 2.59 $\mathrm{g \cdot g^{-1} \cdot h^{-1}}$ for *A. flexus* and *A. arpilobus*, respectively. (2) Significant differences in uptake and allocation among plant organs were observed for different nitrogen sources in both soil layers ($P < 0.05$). The ^{15}N uptake in roots of *A. flexus* was greater than that in roots of *A. arpilobus*, and all three nitrogen forms were primarily allocated to leaves. (3) In different soil layers, the contribution rates of different nitrogen sources to both plants followed the order: ^{15}N-NO$_3{}^-$ > ^{15}N-Glycine > ^{15}N-NH$_4{}^+$. Nitrate nitrogen contributed 37–41% to nitrogen absorption in *A. flexus* and up to 45% in *A. arpilobus*. (4) Significant differences in recovery rates of different nitrogen forms were observed among plant organs ($P < 0.05$). In the 0–5 cm soil layer, recovery rates of nitrate nitrogen in all organs followed the pattern: leaf > stem > root, while in the 5–15 cm layer, *A. flexus* showed the pattern: leaf > root > stem. Overall, in the Gurbantunggut Desert ecosystem, legumes with different life forms exhibit both consistency and divergence in nitrogen uptake and allocation capacity, influenced by soil depth and nitrogen form. These results provide a theoretical basis for understanding nitrogen uptake, utilization, and allocation in legumes in arid and semi-arid regions of Xinjiang.

Keywords: legumes, plant organs, ^{15}N isotopic labeling, nitrogen uptake, Gurbantunggut Desert

Introduction

Nitrogen is the primary element limiting plant growth among all essential nutrients. The source and allocation of nitrogen not only affect nitrogen use efficiency but also alter nitrogen internal cycling and turnover (Hooper & Johnson, 1999; Kou et al., 2015; Tao et al., 2016; Zhou et al., 2018). Numerous studies have shown that the main forms of nitrogen available for plant uptake include nitrate nitrogen, ammonium nitrogen, and organic nitrogen (Wang et al., 2016; Kaur et al., 2016). The forms and quantities of nitrogen absorbed by plants from soil play a crucial role in global nitrogen cycling. However, due to differences in plant biological characteristics, growth environments, and the varying effectiveness of different nitrogen forms on plant growth, the contributions of different nitrogen forms to different plants show significant variation (Hou & Zhuang, 2021). Through long-term adaptation to nitrogen nutritional environments, different plants have developed preferential selectivity for different nitrogen sources, ultimately forming distinct nitrogen utilization and allocation strategies (Tegeder & Masclaux, 2018; Zhuang et al., 2022). Based on preferential absorption and utilization of different nitrogen forms, plants are classified as nitrophilic or ammonophilic (McKane et al., 2002). Some nitrophilic plants such as *Erodium oxyrrhynchum*, *Hyalea pulchella*, *Nonea caspica*, *Lactuca undulata*, *Ceratocarpus arenarius*, and *Suaeda glauca* exhibit strong nitrate nitrogen absorption capacity, growing rapidly under nitrate conditions and showing preferential utilization (Hou, 2022). In contrast, ammonophilic plants such as rice (*Oryza sativa*), masson pine (*Pinus massoniana*), iron fern (*Dicranopteris linearis*), white spruce (*Picea glauca*), and cocklebur (*Xanthium sibiricum*) preferentially absorb and utilize ammonium nitrogen (Fried et al., 1965; Wallander et al., 1997; Lee, 1998; Li et al., 2013; Sun, 2020).

Globally, Leguminosae comprises approximately 765 genera and 19,500 species, widely distributed worldwide (Zhao et al., 2021). China hosts about 184 genera and 1,234 species, found in all provinces and regions (Xiong & Wang, 2018). Legumes are abundant in arid desert ecosystems, serving as central providers of available nitrogen and important pioneer species in these regions (Allen & Allen, 1981). During the 20th century, most research considered only ammonium and nitrate nitrogen as directly absorbable by plants (Schimel & Bennett, 2004). However, with deeper investigation into plant nitrogen nutrition, scholars gradually discovered that plants can also utilize soluble organic nitrogen in soil (Mo et al., 2002; Wang & Liu, 2009). In nitrogen-limited systems such as alpine, boreal, and tundra ecosystems, organic nitrogen absorption represents a crucial pathway (Feng, 2020; Du, 2020). Zhang et al. (2021) found in nitrogen addition experiments with alpine legumes in the eastern Qilian Mountains that nitrogen accumulation in different organs of *Trigonella ruthenica* followed the pattern: stem < flower < leaf. Related studies have confirmed that different plant functional groups exhibit differences in nitrogen uptake characteristics, allocation patterns, and nitrogen transport pathways within plants (Ye et al., 2015; Sun, 2019). Most research on legume nitrogen uptake, utilization, and allocation has focused on agricultural and forest ecosystems (An, 2008; Liu, 2013), while studies on nitrogen absorption and allocation by desert legumes in response to different nitrogen forms remain limited.

Previous studies have found that in the Gurbantunggut Desert, non-leguminous herbaceous plants with different life forms exhibit preferential utilization of different nitrogen forms, which objectively reduces interspecific competition for resources (Zhuang et al., 2020). Additionally, research has shown that four short-lived non-leguminous herbaceous plants in the Gurbantunggut Desert ecosystem display diverse and differentiated nitrogen uptake capacities, and all can absorb soluble organic nitrogen sources from soil (Zhuang & Hou, 2021). Therefore, we pose the scientific question: In the nitrogen-deficient typical temperate desert ecosystem of the Gurbantunggut Desert, what are the absorption and allocation characteristics of different nitrogen forms in various organs of legumes? We hypothesize that different organs of legumes in this region exhibit distinct nitrogen absorption and allocation characteristics for different nitrogen forms and show preferential absorption for specific nitrogen sources. This study selected this typical temperate desert as the experimental area and used two common legume species, *Astragalus flexus* and *Astragalus arpilobus*, as research subjects. Using ^{15}N isotopic tracer technology, we investigated nitrogen uptake and utilization strategies of different nitrogen forms in legumes with different life forms. The results provide a theoretical basis for understanding nitrogen uptake preferences in legumes, thereby enhancing knowledge of nitrogen cycling in desert ecosystems, maintaining nitrogen balance, and offering theoretical support for sustainable nitrogen utilization and development of legumes in arid and semi-arid regions of Xinjiang.

1.1 Study Area and Sample Plot Description

The study area is located in the hinterland of the Eurasian continent (84°31 –90°00 E, 44°11 –46°20 N, elevation 300–600 m), covering approximately 4.88×10^4 km^2, and represents China's largest fixed and semi-fixed desert. The average annual precipitation is 79 mm, with higher precipitation in spring accounting for 47.6% of the annual total. The mean annual temperature is 7.3 °C, and annual evaporation reaches 2,607 mm (Zhou, 2010). Precipitation concentrates between April and July, with minimal rainfall in other months. In winter, the desert surface is typically covered with 20 cm of snow. This precipitation-temperature-time pattern promotes lush herbaceous growth in spring and summer (Wang et al., 2006). The pH of 0–15 cm soil layer in the experimental area ranges from 8.31 ± 0.04 (Akberjan et al., 2022), and the natural water content of 0–20 cm soil layer is 0.061 cm$^3 \cdot$ cm^{-3} (Dong et al., 2017). The desert hosts 208 species of higher plants belonging to 123 genera across 30 families, with the most abundant being Chenopodiaceae (24 genera, 53 species), Brassicaceae (16 genera, 22 species), Asteraceae (15 genera, 20 species), Fabaceae (8 genera, 18 species), Poaceae (12 genera, 13 species), Polygonaceae (3 genera, 11 species), Zygophyllaceae (4 genera, 8 species), and Tamaricaceae (2 genera, 8 species). These eight families comprise 84 genera and 153 species, accounting for approximately 68% of the total genera and 74% of the total species in the desert (Zhang & Chen, 2002). The 18 legume species in the Gurbantunggut Desert play important ecological roles in stabilizing and balancing nutrients in sandy soil systems.

This study utilized the long-term monitoring plot established by our research group and the Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, at the southern edge of the Gurbantunggut Desert (86°13 E, 44°40 N). The plot features a comprehensive long-term meteorological and soil physicochemical property monitoring system. In April 2021, four relatively uniform and flat 10 m × 10 m quadrats were established as replicates, spaced more than 60 m apart. Soil nutrient conditions in the sample plots during the May experimental period are shown in .

Table 1 Soil nutrient status in the Gurbantunggut Desert sample plots (n = 4, mean ± SD)

Soil composition content	Soil depth 0–5 cm	Soil depth 5–15 cm
Soil organic C (g/kg)	0.543 ± 0.076a	0.432 ± 0.029b
Total N (g/kg)	0.172 ± 0.031a	0.074 ± 0.025b
NH_4^+-N ($g \cdot g^{-1}$)	12.533 ± 0.964a	9.287 ± 0.878b
NO_3^--N ($g \cdot g^{-1}$)	25.387 ± 1.589a	20.086 ± 0.457b
Organic N ($g \cdot g^{-1}$)	15.667 ± 0.635a	11.286 ± 1.285b

Note: Different lowercase letters indicate significant differences between soil layers (P < 0.05).

1.2 Plant Selection

Within the selected large quadrats, monospecific communities of *Astragalus flexus* and *Astragalus arpilobus* were identified. Thirty-two 50 cm × 50 cm sub-quadrats were established for each species, totaling 64 plants. Experiments were conducted with two soil depths, four nitrogen form treatments, and four replicates. Based on plant growth status, the ^{15}N isotope addition experiment was performed during the peak biomass period (late May). Two legume species were treated with four different nitrogen forms: ^{15}N-NH_4Cl, ^{15}N-KNO_3, and ^{15}N-Glycine (all labeled nitrogen from Shanghai Research Institute of Chemical Industry) and CK (no ^{15}N labeling). At this growth stage, root systems of both legumes were distributed in the 0–15 cm layer (Akberjan et al., 2022). Therefore, two soil depths were set at 0–5 cm and 5–15 cm, with nitrogen injection depths at 3 cm and 12 cm, respectively. In the nitrogen labeling experiment, each 50 cm × 50 cm sub-quadrat received labeled nitrogen at a standard rate of 0.6 $g \cdot m^{-2}$ (^{15}N abundance >99%), with each of the three nitrogen forms added at 0.2 $g \cdot m^{-2}$. Unlabeled nitrogen served as the CK treatment. To distinguish plant uptake of different nitrogen forms, only one nitrogen form was ^{15}N-labeled per quadrat, while the other two were unlabeled. Since glycine is the predominant amino acid directly absorbable by plants from soil (Bol & Pflieger, 2002), it was used to represent organic nitrogen. The isotopic nitrogen concentrations were $^{15}N\text{-}NH_4^+$ (99.14%), $^{15}N\text{-}NO_3^-$ (99.19%), and ^{15}N-Glycine (99.04%). To ensure uniform distribution within each quadrat, sub-quadrats were divided into 49 small squares (approximately 7.1 cm per side). The nitrogen mixture was completely dissolved in deionized water, and equal volumes (3 mL) were injected at the center of each square using a 5 mL syringe. The nitrogen addition method followed Wang et al. (2016).

1.3 Plant Collection and Analysis

Forty-eight hours after nitrogen application, plants were collected from both control and three ^{15}N-treated quadrats. Within each designated large quadrat, several 50 cm × 50 cm sub-quadrats were selected, and three plant samples were collected from each, with species distinguished. Aboveground biomass was harvested using the clipping method, separating stems and leaves. Root biomass was excavated from sub-quadrats, collecting the most complete underground portions possible. Whole plants with ^{15}N addition were removed and rinsed with deionized water to remove surface soil. Roots were soaked in 0.5 $mol \cdot L^{-1}$ $CaCl_2$ solution for 0.5 h to remove adsorbed ^{15}N, followed by distilled water rinsing. In the laboratory, plants were separated into roots, stems, and leaves (Guo et al., 2005). Above- and belowground parts were dried in an electric thermostatic drying oven (GZX-9076MBE, Shanghai Boxun Medical Equipment Factory) at 70 °C for 48 h to constant weight and then weighed. Roots, stems, and leaves of both species were ground into powder using a Retsch RS200 vibratory disc mill (Lu et al., 2000). Samples of 2 mg were weighed using a 0.001 g precision balance. Unlabeled nitrogen quadrats served as controls for natural abundance. Nitrogen content and plant ^{15}N content were analyzed using a stable isotope mass spectrometer (MAT253-SN08867G, USA). Calculation methods followed Clemmensen et al. (2008), Jacob & Leuschner (2015), Wang et al. (2016), and Meng (2016).

The calculations are as follows:

- atom% excess = atom% labeled – atom% control
- Ulabeled = atom% excess × Ncontent × M
- Uunlabeled = Ulabeled × (munlabeled / mlabeled)
- Nuptake = Uunlabeled / (MGBG × H)
- $T^{15}Nuptake = {}^{15}Nuptake(NH_4^+) + {}^{15}Nuptake(NO_3^-) + {}^{15}Nuptake(Glycine)$

- RecoveryplantN(%) = Ulabeled / ^{15}Nadded × 100
- RN from (%) = RNH$_4{}^+$/NO$_3{}^-$/Glycine / RTN

Where: atom% excess represents plant absorption using ^{15}N atomic percent excess; atom% labeled denotes the atomic percent concentration of ^{15}N in labeled plants; atom% control represents the atomic percent concentration in CK plants; Ulabeled is plant ^{15}N uptake ($g \cdot m^{-2}$); Ncontent refers to plant nitrogen concentration; M is plant biomass (g); Uunlabeled represents plant N uptake ($g \cdot m^{-2}$); munlabeled is the native soil nitrogen concentration in control plots ($g \cdot g^{-1}$); mlabeled is the total ^{15}N addition to soil ($g \cdot g^{-1}$); Nuptake is plant nitrogen absorption rate ($g \cdot g^{-1} \cdot h^{-1}$), calculated as Uunlabeled divided by belowground biomass (MGBG) and labeling time (H); TNuptake is the sum of absorption rates for different nitrogen forms; RecoveryplantN (%) indicates the recovery rate of isotopic ^{15}N in plants (%); ^{15}Nadded is the amount of ^{15}N added per square meter; RN from (%) represents the contribution rate of different ^{15}N sources; RNH$_4{}^+$/NO$_3{}^-$/Glycine is the recovery rate of a single ^{15}N form; and RTN is the sum of recovery rates for the three nitrogen forms.

1.4 Data Analysis

Data were organized using Excel 2021 and statistically analyzed using SPSS 23.0. One-way ANOVA was employed for multiple comparisons of differences in nitrogen uptake-related indicators among plant organs under the influence of different soil layers and species. Multi-way ANOVA was used to verify the effects of different factors (species, nitrogen form, soil depth) on ^{15}N uptake and allocation in organs of legumes with different life forms, considering interactive effects among factors. Origin 2021 software was used for figure preparation.

2.1 Nitrogen Uptake Rates of Two Legume Species for Different Nitrogen Forms

As shown in [Figure 1: see original paper], the ^{15}N uptake rates of the two legume species for different nitrogen forms varied across soil layers. In the 0–5 cm layer, both species showed significant differences in uptake rates among the three nitrogen forms ($P < 0.05$), with the trend: nitrate nitrogen > glycine > ammonium nitrogen. *Astragalus flexus* exhibited uptake rates of 2.07, 3.06, and 2.45 $g \cdot g^{-1} \cdot h^{-1}$ for ammonium, nitrate, and glycine, respectively, with nitrate uptake being 1.48 and 1.25 times higher than ammonium and glycine, respectively. *Astragalus arpilobus* showed uptake rates of 0.88, 2.59, and 1.54 $g \cdot g^{-1} \cdot h^{-1}$ for the three forms, with nitrate uptake 2.95 and 1.68 times higher than ammonium and glycine, respectively. Total ^{15}N uptake rates were 7.58 and 5.01 $g \cdot g^{-1} \cdot h^{-1}$ for *A. flexus* and *A. arpilobus*, respectively.

In the 5–15 cm layer, both species again showed significant differences among nitrogen forms ($P < 0.05$), maintaining the same trend: nitrate > glycine > ammonium. *A. flexus* exhibited uptake rates of 1.94, 3.26, and 2.23 $g \cdot g^{-1} \cdot h^{-1}$, with nitrate uptake 1.68 and 1.46 times higher than ammonium and glycine, respectively. *A. arpilobus* showed rates of 0.81, 1.90, and 1.29 $g \cdot g^{-1} \cdot h^{-1}$, with nitrate uptake 2.35 and 1.47 times higher than ammonium and glycine, respectively.

Overall, both species exhibited the highest absorption rates for nitrate nitrogen, followed by glycine, with ammonium nitrogen showing the lowest rates. Total ^{15}N uptake rates were 7.43 and 4.00 $g \cdot g^{-1} \cdot h^{-1}$ for *A. flexus* and *A. arpilobus*, respectively. As soil depth increased, *A. flexus* showed decreased uptake rates for ammonium nitrogen and glycine but increased nitrate uptake. In contrast, *A. arpilobus* exhibited decreased uptake rates for all three nitrogen forms with increasing soil depth, with nitrate showing the most pronounced decline. In the 0–5 cm and 5–15 cm layers, *A. flexus* uptake rates ranged 1.94–2.07, 3.06–3.26, and 2.23–2.45 $g \cdot g^{-1} \cdot h^{-1}$ for ammonium, nitrate, and glycine, respectively, while *A. arpilobus* rates ranged 0.81–0.88, 1.90–2.59, and 1.29–1.54 $g \cdot g^{-1} \cdot h^{-1}$.

Note: Panels A and B represent 0–5 cm and 5–15 cm soil layers, respectively; the same applies below. Different lowercase letters indicate significant differences among three nitrogen forms for the same species, while different uppercase letters indicate significant differences between species for the same nitrogen form ($P < 0.05$).

2.2 Organ-Specific Uptake and Distribution of Different Nitrogen Forms in Two Legume Species

As shown in [Figure 2: see original paper], the ^{15}N uptake and distribution in different organs of *A. flexus* varied across soil layers. In the 0–5 cm layer, both species showed the same trend for uptake and accumulation: nitrate > glycine > ammonium, with leaf accumulation significantly greater than stems and roots. For *A. flexus*, nitrate uptake and distribution were highest: root (49.54 $g \cdot m^{-2}$) < stem (53.29 $g \cdot m^{-2}$) < leaf (104.66 $g \cdot m^{-2}$). Root nitrate uptake was 1.34 and 1.21 times higher than ammonium and glycine, respectively; stem nitrate accumulation was 3.13 and 1.25 times higher; and leaf nitrate accumulation was 1.39 and 1.06 times higher. For *A. arpilobus*, nitrate uptake and distribution were also highest: root (3.03 $g \cdot m^{-2}$) < stem (35.73 $g \cdot m^{-2}$) < leaf (51.46 $g \cdot m^{-2}$). Root nitrate uptake was 1.91 and 1.53 times higher than ammonium and glycine; stem nitrate accumulation was 2.48 and 1.52 times higher; and leaf nitrate accumulation was 1.50 and 1.14 times higher. Total ^{15}N content across organs was: stem (113.07 $g \cdot m^{-2}$) < root (127.46 $g \cdot m^{-2}$) < leaf (278.03 $g \cdot m^{-2}$) for *A. flexus*, and root (6.58 $g \cdot m^{-2}$) < stem (73.41 $g \cdot m^{-2}$) < leaf (171.32 $g \cdot m^{-2}$) for *A. arpilobus*.

In the 5–15 cm layer, *A. flexus* again showed highest nitrate uptake: stem

(47.98 $g \cdot m^{-2}$) < root (48.69 $g \cdot m^{-2}$) < leaf (104.12 $g \cdot m^{-2}$). Root nitrate uptake was 1.41 and 1.48 times higher than ammonium and glycine; stem nitrate accumulation was 2.64 and 1.03 times higher; and leaf nitrate accumulation was 1.55 and 1.13 times higher. For *A. arpilobus*, nitrate uptake was highest: root (2.87 $g \cdot m^{-2}$) < stem (31.88 $g \cdot m^{-2}$) < leaf (57.67 $g \cdot m^{-2}$). Root nitrate uptake was 1.89 and 1.08 times higher than ammonium and glycine; stem nitrate accumulation was 2.55 and 1.70 times higher; and leaf nitrate accumulation was 1.36 and 1.03 times higher. Total ^{15}N content was: root (112.89 $g \cdot m^{-2}$) < stem (116.20 $g \cdot m^{-2}$) < leaf (263.58 $g \cdot m^{-2}$) for *A. flexus*, and root (7.05 $g \cdot m^{-2}$) < stem (63.10 $g \cdot m^{-2}$) < leaf (156.19 $g \cdot m^{-2}$) for *A. arpilobus*.

Overall, *A. flexus* more readily absorbed and accumulated nitrogen than *A. arpilobus*, indicating different nitrogen utilization and allocation strategies between legumes with different life forms. Multi-factor interactions significantly affected nitrogen uptake and allocation (see), with species × nitrogen form, species × organ, organ × nitrogen form, and species × organ × soil depth all showing significant effects ($P < 0.001$). This demonstrates that different life forms exhibit significant differences in nitrogen absorption and allocation, which are also influenced by soil depth.

Note: Different lowercase letters indicate significant differences among three nitrogen forms within the same organ, while different uppercase letters indicate significant differences among organs for the same nitrogen form (P < 0.05).

Table 2 Multi-way ANOVA analysis of effects of species, nitrogen form, soil depth, organ, and their interactions on nitrogen uptake and distribution in plant organs

Effect	P-value
Species	< 0.001
Organ	< 0.001
Soil depth	< 0.001
Nitrogen form	< 0.001
Species × Organ	< 0.001
Species × Soil depth	< 0.001
Species × Nitrogen form	< 0.001
Organ × Soil depth	< 0.001
Organ × Nitrogen form	< 0.001
Soil depth × Nitrogen form	< 0.001
Species × Organ × Soil depth	< 0.001
Species × Organ × Nitrogen form	< 0.001
Species × Soil depth × Nitrogen form	< 0.001
Organ × Soil depth × Nitrogen form	< 0.001
Species × Organ × Soil depth × Nitrogen form	< 0.001

Note: P-values for significant effects and interactions are shown in bold (P <

0.001).

2.3 Recovery Rates and Contribution Rates of Different Nitrogen Forms in Two Legume Species and Their Organs

Nitrogen directly affects organ-specific nitrogen absorption and allocation characteristics, while recovery rates reflect nitrogen distribution among organs. As shown in [Figure 3: see original paper], recovery rates of three nitrogen forms varied across organs of both legume species. In the 0–5 cm layer, *A. flexus* leaves showed the highest recovery rates for all three nitrogen forms, significantly greater than roots and stems ($P < 0.05$). For ammonium nitrogen, recovery rates in *A. flexus* organs were: stem (16%) < root (25%) < leaf (70%). For nitrate nitrogen: root (35%) < stem (49%) < leaf (86%). For glycine: root (27%) < stem (39%) < leaf (81%). For *A. arpilobus*, recovery rates across organs for all nitrogen forms followed: root < stem < leaf, with nitrate showing the highest recovery, followed by glycine, and ammonium the lowest. Leaf recovery rates for ammonium, nitrate, and glycine were 34%, 51%, and 45%, respectively.

In the 5–15 cm layer, recovery rates in *A. flexus* organs were significantly higher than in *A. arpilobus* ($P < 0.05$). For *A. flexus*, recovery rates across organs followed: stem < root < leaf, with nitrate > glycine > ammonium. Stem recovery rates for ammonium, nitrate, and glycine were 17%, 27%, and 22%, respectively. For *A. arpilobus*, recovery rates were: ammonium: root (1%) < stem (11%) < leaf (39%); nitrate: root (3%) < stem (29%) < leaf (52%); glycine: root (2%) < stem (17%) < leaf (51%).

As shown in [Figure 4: see original paper], the contribution rates of three ^{15}N forms to both species 48 h after application varied by soil layer. In the 0–5 cm layer, contributions to *A. flexus* were 25% (^{15}N-NH_4^+), 41% (^{15}N-NO_3^-), and 34% (^{15}N-Glycine), while contributions to *A. arpilobus* were 23%, 45%, and 32%, respectively. This indicates that ^{15}N-NH_4^+ contribution to *A. flexus* was higher than to *A. arpilobus*, while ^{15}N-NO_3^- contribution was lower. In the 5–15 cm layer, contributions to *A. flexus* were 30% (^{15}N-NH_4^+), 37% (^{15}N-NO_3^-), and 33% (^{15}N-Glycine), showing that ^{15}N-NH_4^+ contribution increased with soil depth for *A. flexus*, while ^{15}N-NO_3^- contribution decreased. For *A. arpilobus*, contributions were 23% (^{15}N-NH_4^+), 43% (^{15}N-NO_3^-), and 34% (^{15}N-Glycine), indicating that soil depth did not affect ^{15}N-NH_4^+ contribution to *A. arpilobus*, while ^{15}N-NO_3^- contribution decreased and ^{15}N-Glycine increased with depth.

Note: Different lowercase letters indicate significant differences among three nitrogen forms within the same organ, while different uppercase letters indicate significant differences among organs for the same nitrogen form ($P < 0.05$).

Discussion and Conclusion

This study used ^{15}N isotopic tracer methodology to investigate nitrogen uptake rates, contribution rates, and organ-specific absorption, allocation, and recovery of three nitrogen forms applied at different soil depths to two legume species with different life forms in the Gurbantunggut Desert. Across soil layers, both species showed consistent preferences, with the highest absorption rates for nitrate nitrogen, followed by glycine, and lowest for ammonium nitrogen, indicating a preference for nitrate. Zhuang and Hou (2021) found that four ephemeral plants in the Gurbantunggut Desert showed higher nitrate absorption rates in the 0–5 cm layer than in the 5–10 cm layer, consistent with our results. Hou and Zhuang (2021) reported that two non-leguminous desert ephemeral plants showed higher absorption rates for ammonium, nitrate, and organic nitrogen in the 0–5 cm layer than in the 5–15 cm layer, with nitrate showing the highest rate, aligning with our findings. Many studies indicate that legumes often maintain high NO_3^- concentrations in xylem sap, facilitating NO_3^- absorption (Arndt et al., 2004). Additionally, nitrate's mobility in soil may contribute to rapid plant absorption (Li et al., 2015). As shown in , the 0–5 cm soil layer in the Gurbantunggut Desert has relatively high nutrient content, with available nitrogen sources following the proportion: $N\text{-}NO_3^- > N\text{-Glycine} > N\text{-}NH_4^+$. We hypothesize that the strong nitrate absorption capacity likely relates to high soil nitrate content in the study area, with plants preferentially absorbing nitrogen from the 0–5 cm layer, consistent with Hou (2022). Sun (2020) found that nitrogen uptake rates in two legume seedlings (*Melilotus albus* and *M. officinalis*) were 3–4 times higher than in our experiment, possibly due to differences in species, soil nutrient environment, nitrogen application rate, and habitat conditions.

Across soil layers, both species showed significant differences in organ-specific uptake and allocation of three nitrogen forms ($P < 0.05$). Liu et al. (2015) previously concluded that plant roots primarily absorb inorganic nitrogen, but our results show that organic nitrogen (glycine) uptake and rates exceeded those of ammonium nitrogen, indicating strong organic nitrogen absorption capacity in legumes within the experimental timeframe. One reason may be that rhizobia form nodules on legume roots, reducing N_2 to ammonia and providing nitrogen for the plant. Rhizobial root morphology significantly influences organic nitrogen absorption, suggesting that mycorrhizae may enhance legume capacity to absorb organic nitrogen (Cao et al., 2015), consistent with our findings. Generally, ATP consumption for nitrate absorption exceeds that for ammonium absorption (Wang & Macko, 2011). Soil ammonium is converted to glutamate after root absorption, directly utilizable by plant organs, whereas nitrate absorption requires greater ATP expenditure (Templer & Dawson, 2004). Our study found that different ^{15}N forms accumulated primarily in leaves, likely related to total nitrogen content and recovery rates in legume leaves, which may be positively correlated, leading to differential nitrogen allocation among organs, consistent with Gao et al. (2017). Previous studies indicate most plants prefer nitrate over ammonium (Hou, 2022), and our results align with this view. The effective root systems of both species were distributed in shallow soil, with *A. flexus* having thicker roots, more fibrous roots, and greater stem and leaf numbers than *A. arpilobus*, leading to inconsistent nitrogen absorption and allocation patterns between the two species. Overall, shows that species, soil depth, and nitrogen source all influence organ-specific nitrogen uptake and allocation.

Recovery rates of different ^{15}N forms in plant organs directly indicate the proportion of nitrogen in a given organ (Li, 2018). Across soil layers, both species preferred nitrate recovery, with organ recovery rates following: ^{15}N-NO_3^- > ^{15}N-Glycine > ^{15}N-NH_4^+, and leaf recovery rates exceeding other organs. This demonstrates both differential and consistent nitrogen recovery capacities among plants, similar to Xiao et al. (2022). Liu et al. (2018) found that in alpine artificial grasslands, stem and leaf recovery rates exceeded root rates when nitrogen fertilizer was applied to the soil surface (0 cm), consistent with our results. Comparatively across soil layers, *A. flexus* showed recovery rates of stem < root < leaf for all nitrogen forms, while *A. arpilobus* showed root < stem < leaf. Xu et al. (2018) reported recovery rates in stem-leaf portions exceeding roots for three ^{15}N forms, with stem-leaf recovery reaching 22%, 44%, and 29% for ^{15}N-NH_4^+, ^{15}N-NO_3^-, and ^{15}N-Glycine, respectively ($P < 0.05$), similar to our findings. Freschet et al. (2010) found that roots could resorb nitrogen during senescence in 40 perennial herb species from subarctic regions, with a nitrogen recovery rate of 27%, similar to root recovery rates in *A. flexus*. Generally, perennial herb stems contain substantial chlorophyll and participate in photosynthesis (Smith, 1950), explaining why *A. flexus* stem ^{15}N recovery exceeds that of *A. arpilobus*. Zhang et al. (2014) confirmed no significant difference in nitrogen recovery rates between nitrogen-fixing and non-fixing woody plants in Beijing's Dongling Mountains, a finding inconsistent with our results and those of Zhuang & Hou (2021), possibly due to species and habitat differences.

In the same habitat of the Gurbantunggut Desert, different nitrogen sources applied at the same soil depth showed differential contribution rates to different plant species (Hou & Zhuang, 2021). Our results show that nitrate contributed most to both species across soil layers, reaching 41% and 37% for *A. flexus* and 45% and 43% for *A. arpilobus* in the 0–5 cm and 5–15 cm layers, respectively. Nitrate contribution was greater for *A. arpilobus* than *A. flexus* across soil layers, while ammonium contribution to *A. flexus* and glycine contribution to *A. arpilobus* increased with soil depth. Nitrate content absorbed by both species was proportional to its contribution rate, and organic nitrogen sources were absorbed from soil to contribute to plant growth. Generally, higher soil content and proportion of a nitrogen form correspond to higher plant absorption, preference, and contribution rates, consistent with Sun (2020). Hou & Zhuang (2021) found that contributions of ammonium, nitrate, and organic nitrogen to two non-leguminous desert ephemeral plants were greater in the 5–15 cm layer than in the 0–5 cm layer 24 h after ^{15}N addition, similar to our results. However, our study found nitrate contributed most to both legumes, and glycine contributed more than ammonium, possibly related to nitrogen addition timing, species type, and soil depth. Ren et al. (2022) reported that under natural conditions in a 10-year nitrogen addition experiment in Inner Mongolia typical steppe, nitrate contribution was four times that of ammonium, inconsistent with our results, likely due to differences in ecosystem type and soil available nitrogen content between study areas.

Legumes with different ecological niches not only reduce interspecific competition for survival but also significantly improve efficient utilization of soil nitrogen resources, clarifying how legumes and their organs efficiently utilize and allocate limited nitrogen sources in typical temperate desert ecosystems where nitrogen is the second limiting factor. Interspecific competition for soil nitrogen sources is a key factor limiting productivity in temperate desert ecosystems. Therefore, investigating plant preferences for different nitrogen forms, recovery rates, allocation mechanisms, and nitrogen source contribution rates is important for optimizing species composition in desert ecosystems, guiding fertilization in poor soils, and improving nitrogen resource effectiveness. In summary, as a perennial short-lived legume, *A. flexus* has more developed organs than the annual short-lived *A. arpilobus*, likely causing differences in organ-specific nitrogen absorption and allocation strategies between the two species. However, the factors influencing organ-specific nitrogen absorption, allocation, and utilization in desert ecosystems remain uncertain and may relate to geographic and soil environments, soil microbial communities, and other factors in arid regions, requiring further investigation combined with soil temperature, moisture, and environmental physicochemical factors. Clearly, nitrogen deficiency characterizes the Gurbantunggut Desert ecosystem, where legumes regulate their preferential nitrogen absorption and organ allocation through long-term adaptation to soil nitrogen forms to complete their life cycles.

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